

09/26/00
JC759 U.S. PTO

39-27-67

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I, hereby certify that the below identified application and attached documents, pursuant to 37 C.F.R. §1.10, are being deposited as "Express Mail" this date with the United States Postal service in an envelope addressed to Box PATENT APPLICATION, Commissioner for Patents, Washington, D.C. 20231, on

Date: September 26, 2000

By Dawn R. Lovi
Dawn R. Lovi

JC658 U.S. PTO
09/670421
09/26/00

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Docket No.: 40224.00001

Box PATENT APPLICATION
Commissioner for Patents
Washington, D.C. 20231

NEW UTILITY PATENT APPLICATION

Commissioner:

Herewith is the utility patent application of Applicant:
Dale WALLIS and James L. WALLIS

TITLE: DETECTION PREVENTION AND TREATMENT OF PAPILLOMATOUS DIGITAL DERMATITIS

Including:

- ☐ Declaration
- ☒ Abstract
- 37 Pages of Specification (only specification and claims)
- 17 Numbered claims(s)
- 7 Sheets of drawing: ☒ informal ☐ formal
- ☐ This is a filing under 37 C.F.R. §1.47 (Petition attached)
- ☐ Attached is an Assignment to _____
- ☐ Attached: Assignment Recordation Cover Sheet
- ☐ Priority is hereby claimed under 37 C.F.R. §1.55 and 35 U.S.C. §119 based on prior foreign application(s) No(s). _____ filed in _____ on _____. Certified copy (copies) ☐ attached; ☐ already filed on _____ in U.S. Application No. _____, filed _____.
- ☐ Oath or Declaration from a prior application [for continuation/divisional with below request checked]
- ☒ This is a request for filing a divisional application under 37 C.F.R. 1.53(b), of U.S. Patent Application No. 08/903,559. Prior application information: Examiner: Mark Navarro; Group/Art Unit: 1645. The entire disclosure of the prior application, from which an oath or declaration is supplied under Box 4b, is considered a part of the disclosure of the

- | | 1990 | 1991 | 1992 | 1993 | 1994 | 1995 | 1996 | 1997 | 1998 | 1999 | 2000 | 2001 | 2002 | 2003 | 2004 | 2005 | 2006 | 2007 | 2008 | 2009 | 2010 | 2011 | 2012 | 2013 | 2014 | 2015 | 2016 | 2017 | 2018 | 2019 | 2020 | 2021 | 2022 | 2023 | 2024 | 2025 | 2026 | 2027 | 2028 | 2029 | 2030 | 2031 | 2032 | 2033 | 2034 | 2035 | 2036 | 2037 | 2038 | 2039 | 2040 | 2041 | 2042 | 2043 | 2044 | 2045 | 2046 | 2047 | 2048 | 2049 | 2050 | 2051 | 2052 | 2053 | 2054 | 2055 | 2056 | 2057 | 2058 | 2059 | 2060 | 2061 | 2062 | 2063 | 2064 | 2065 | 2066 | 2067 | 2068 | 2069 | 2070 | 2071 | 2072 | 2073 | 2074 | 2075 | 2076 | 2077 | 2078 | 2079 | 2080 | 2081 | 2082 | 2083 | 2084 | 2085 | 2086 | 2087 | 2088 | 2089 | 2090 | 2091 | 2092 | 2093 | 2094 | 2095 | 2096 | 2097 | 2098 | 2099 | 2100 | 2101 | 2102 | 2103 | 2104 | 2105 | 2106 | 2107 | 2108 | 2109 | 2110 | 2111 | 2112 | 2113 | 2114 | 2115 | 2116 | 2117 | 2118 | 2119 | 2120 | 2121 | 2122 | 2123 | 2124 | 2125 | 2126 | 2127 | 2128 | 2129 | 2130 | 2131 | 2132 | 2133 | 2134 | 2135 | 2136 | 2137 | 2138 | 2139 | 2140 | 2141 | 2142 | 2143 | 2144 | 2145 | 2146 | 2147 | 2148 | 2149 | 2150 | 2151 | 2152 | 2153 | 2154 | 2155 | 2156 | 2157 | 2158 | 2159 | 2160 | 2161 | 2162 | 2163 | 2164 | 2165 | 2166 | 2167 | 2168 | 2169 | 2170 | 2171 | 2172 | 2173 | 2174 | 2175 | 2176 | 2177 | 2178 | 2179 | 2180 | 2181 | 2182 | 2183 | 2184 | 2185 | 2186 | 2187 | 2188 | 2189 | 2190 | 2191 | 2192 | 2193 | 2194 | 2195 | 2196 | 2197 | 2198 | 2199 | 2200 | 2201 | 2202 | 2203 | 2204 | 2205 | 2206 | 2207 | 2208 | 2209 | 2210 | 2211 | 2212 | 2213 | 2214 | 2215 | 2216 | 2217 | 2218 | 2219 | 2220 | 2221 | 2222 | 2223 | 2224 | 2225 | 2226 | 2227 | 2228 | 2229 | 2230 | 2231 | 2232 | 2233 | 2234 | 2235 | 2236 | 2237 | 2238 | 2239 | 2240 | 2241 | 2242 | 2243 | 2244 | 2245 | 2246 | 2247 | 2248 | 2249 | 2250 | 2251 | 2252 | 2253 | 2254 | 2255 | 2256 | 2257 | 2258 | 2259 | 2260 | 2261 | 2262 | 2263 | 2264 | 2265 | 2266 | 2267 | 2268 | 2269 | 2270 | 2271 | 2272 | 2273 | 2274 | 2275 | 2276 | 2277 | 2278 | 2279 | 2280 | 2281 | 2282 | 2283 | 2284 | 2285 | 2286 | 2287 | 2288 | 2289 | 2290 | 2291 | 2292 | 2293 | 2294 | 2295 | 2296 | 2297 | 2298 | 2299 | 2300 | 2301 | 2302 | 2303 | 2304 | 2305 | 2306 | 2307 | 2308 | 2309 | 2310 | 2311 | 2312 | 2313 | 2314 | 2315 | 2316 | 2317 | 2318 | 2319 | 2320 | 2321 | 2322 | 2323 | 2324 | 2325 | 2326 | 2327 | 2328 | 2329 | 2330 | 2331 | 2332 | 2333 | 2334 | 2335 | 2336 | 2337 | 2338 | 2339 | 2340 | 2341 | 2342 | 2343 | 2344 | 2345 | 2346 | 2347 | 2348 | 2349 | 2350 | 2351 | 2352 | 2353 | 2354 | 2355 | 2356 | 2357 | 2358 | 2359 | 2360 | 2361 | 2362 | 2363 | 2364 | 2365 | 2366 | 2367 | 2368 | 2369 | 2370 | 2371 | 2372 | 2373 | 2374 | 2375 | 2376 | 2377 | 2378 | 2379 | 2380 | 2381 | 2382 | 2383 | 2384 | 2385 | 2386 | 2387 | 2388 | 2389 | 2390 | 2391 | 2392 | 2393 | 2394 | 2395 | 2396 | 2397 |
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THE FOLLOWING FILING FEE IS BASED ON CLAIMS AS FILED
Per MPEP §§506 AND 607

	Claims filed		Basic claims		Present Extra		Fees
Basic Fee:							\$ 690.00
Total Effective Claims	8	minus	20	=		x \$ 18.00 =	.00
Independent Claims	6	minus	3	=	3	x \$ 78.00	234.00
						Subtotal =	\$924.00
If "small entity" status box above is checked, enter half (1/2) of Subtotal and subtract							462.00
TOTAL FILING FEE							= \$462.00
If "assignment" box is checked, add recording fee:						+ \$ 40.00	
If "37 C.F.R. §1.47" box above is checked, add Petition fee (per 37 C.F.R. §1.17(h)):						+ \$130.00	
TOTAL FEE							= \$462.00


☐ A check in the amount of \$_____ is enclosed.

☒ Please charge Deposit Account No. 07-1853 in the amount of \$ 462.00. A duplicate copy of this sheet is attached.

This statement does NOT authorize charge of the issue fee. The Commissioner is hereby authorized to charge any fee specifically authorized hereafter, or any deficiency in the fee(s) filed, or asserted to be filed, or which should have been filed herewith or concerning any paper filed hereafter, and which may be required under 37 C.F.R. §§1.16-1.18 (deficiency only) now or hereafter relative to this Application and the resulting Official document under 37 C.F.R. §1.20, or credit any overpayment to Account No. 07-1853 for which purpose a duplicate copy of this sheet is attached.

Respectfully submitted,

By


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Express Mail No.: EJ194928307US
PATENT
40224.00001

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Dale WALLIS and James L. WALLIS Serial No.: Currently unknown Parent Application 08/903,559 Filing Date: July 31, 1997 Divisional Application Filing Date: September 26, 2000 For: DETECTION PREVENTION AND TREATMENT OF PAPILLOMATOUS DIGITAL DERMATITIS	
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PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

Prior to examination of the above-identified divisional application, which was based upon US Application Serial No. 08/903,339, please amend the application as follows:

IN THE SPECIFICATION:

Please amend the specification as follows:

Page 4, lines 2-5, please replace "Hygieia.....95776" with --American Type Culture Collection, 10801 University Boulevard, Manassas, VA 20110-2209--

IN THE CLAIMS:

Please cancel Claims 1-11 without prejudice. In addition, please add the following new Claims 18 and 19.

--18. A pharmaceutical composition for the prevention and treatment of Papillomatous Digital Dermatitis in ruminants comprising:

a therapeutically effective amount of at least one member selected from the group consisting of bacterial species belonging to the genus *Serpens*;

an immunologically active portion thereof; and

an antigenic epitope cross-reactive with the *Serpens* genera in combination with a veterinarily acceptable diluent or a carrier.

19. A method of preventing or treating Papillomatous Digital Dermatitis in ruminants comprising:

administering to a ruminant a therapeutically effective amount of a combination of at least one member selected from the group consisting of bacterial species belonging to the genus *Serpens*; and

an antigenic epitope cross-reactive with *Serpens* genera.--

REMARKS

This is a Preliminary Amendment in a divisional application, which is based upon a US Application Serial No. 08/903,559. By virtue of this Preliminary Amendment, Claims 12-19 are pending. An examination on the merits of the application is respectfully requested.

The Commissioner is authorized to charge any shortage in the fees due in connection with the filing of this paper, including extension of time fees, to Deposit Account No. 07-1853.

[Signature]

David B. Abel
Attorney for Applicant(s)
Registration No. 32,394

[illegible]

Detection of and Methods and Composition for Prevention
and/or Treatment of
Papillomatous Digital Dermatitis

Field of the Invention

The present invention relates to methods and compositions useful in the detection, prevention and/or treatment of papillomatous digital dermatitis in ruminants and to a novel strain of Serpens spp. bacteria useful for that purpose.

Background of the Invention

Papillomatous Digital Dermatitis (PDD) is a chronic infectious and apparently contagious disease of the feet and/or lower legs of cattle. The disease is known by several common and scientific names including digital dermatitis, interdigital papillomatosis, digital papillomatosis, verrucous dermatitis, footwarts, hairy footwarts, hairy heelwarts, raspberry heel, strawberry foot disease and strawberry footrot. It has been identified as one of the most significant diseases facing the dairy industry today. The disease results in lameness which leads to economically significant reductions in milk production and concomitant declines in animal health such as body weight loss, and fertility. It is believed that the agent can be brought onto a dairy via introduction of new stock or formite transmission from hoof trimmers, dairy testers,

muddy boots on veterinarians, etc. In dairies currently experiencing losses from the disease, it has been estimated that the cost in lost milk production, reproductive losses and increased culling averages at least \$100 per day.

To date, only two control methods have shown promise: use of antibiotics topically (cleaning, curettage and bandaging each foot) or parenterally (problematic for reasons of milk withdrawal), and the use of bacteriocidal footbaths (antibiotics, formaldehyde, iodine, etc.). Although many lesions may respond well to antibiotics or footbaths containing antibacterial compounds, recurrences are known to occur and some evidence suggests development of antibiotic resistance. These approaches are labor intensive, prone to human error, costly (antibiotics for 30 days of footbaths runs \$2,500 for a 400 cow dairy), subject to governmental restrictions, and do not confer either environmental cleanup or lasting protection from recurrence.

In view of the significant economic damage caused by PDD, an effective way to detect and treat animals infected with the disease, as well as a means to protect them against future infection, is highly desirable.

Summary of the Invention

The present invention relates to compositions and methods for the detection, prevention and/or treatment of Papillomatous Digital Dermatitis in ruminants.

The present invention now provides a method of preventing and/or treating Papillomatous Digital Dermatitis

in ruminants comprising administering to the ruminant a therapeutically effective amount of Serpens spp. bacteria or Serpens spp. bacterin and/or an immunologically active portion thereof and/or an antigenic epitope cross-reactive with Serpens spp..

The present invention further comprises a pharmaceutical composition for preventing and/or treating Papillomatous Digital Dermatitis in ruminants comprising a therapeutically effective amount of Serpens spp. bacteria or Serpens spp. bacterin and/or an immunologically active portion thereof and/or an antigenic epitope cross-reactive with Serpens spp. and a veterinerally acceptable diluent or carrier.

The present invention further comprises a method of determining the presence of PDD antibodies in a sample of ruminant serum comprising contacting the sample with an antigen selected from the group consisting of bacteria or bacterin of the Serpens genus or an immunologically active portion thereof and/or an antigenic epitope cross-reactive with Serpens spp. and detecting antibodies in the sample which bind to the antigen. The present invention can also be used to determine the presence of PDD antigen or anti-Serpens spp. antibodies.

The present invention further comprises the use of a diagnostic kit for determining the presence of PDD antibodies wherein the kit comprises an antigen and one or more binding partners.

The present invention also provides biologically pure

Serpens spp. strain HBL-112, and biologically pure Serpens spp. strain HBL-112 bacterin. Serpens spp. strain HBL-112 bacteria Deposit No. 1 and Serpens spp. strain HBL-112 bacterin Deposit No. 2 has been deposited at Hygieia Biological Laboratories at Post Office Box 8300, Woodland, California, 95776. Serpens spp. strain HBL-112 is a member of the Serpens spp. genus and has the biological and morphological characteristics defined below.

Brief description of the Drawings

The present invention is illustrated by the following drawings, in which:

Figure 1 is a graph showing the clinical reduction in total footwart area seen in dairy cattle with preexisting footwarts in response to vaccination using Serpens spp. strain HBL-112 bacterin, according to the present invention as compared to a control. Wart area is given in square centimeters, calculated from millimeter measurements made in two dimensions of the lesions on the feet of clinically affected cattle. Time is shown as days after enrollment in the trial. Total elapsed time is 76 days;

Figure 2 is an interaction bar chart of the wart area by initial and postvaccination measurements (at day 49) in vaccinates and controls, with the associated Games-Howell post-hoc analysis demonstrating significance of the effect of vaccination;

Figure 3 is an interaction bar chart and Games-Howell analysis for serological titers in a random sample of about

half of the enrolled animals by initial and postvaccination measurements (at day 49) in vaccinates and controls;

Figure 4a is a graph showing a typical standard curve of a positive bovine serum sample titrated in a capture ELISA;

Figure 4b is a graph showing a standard curve of a competition ELISA using constant antibody and titrated antigen;

Figure 5 is a photomicrograph illustrating the inducible spiral form of Serpens spp., strain HBL-112;

Figure 6 is a photomicrograph of the same pure culture Serpens spp. strain HBL-112 as Figure 5 showing the long rods, short rods and spherical bodies typical of the Serpens genus; and

Figure 7 is a photomicrograph of the same pure culture of Serpens spp. strain HBL-112 as Figure 5 showing flagella associated with Serpens spp. strain HBL-112.

Detailed Description of the Invention

Isolation and Purification of Serpens spp. strain HBL-112

Prior to the present invention, the only known species of the genus Serpens was Serpens flexibilis which was isolated from the upper centimeters of sediment (mud) found in eutrophic freshwater ponds. S. flexibilis are rod-shaped cells, 0.3-0.4 μm wide by 8-12 μm long. They occur singly or in pairs. Cells in the stationary phase of growth are longer and often possess blebs or spherical protuberances. S. flexibilis has a uniquely flexible motion. They possess

bipolar tufts of 4-10 flagella and also a few lateral flagella. None of the published literature on this organism indicates any proclivity for pathogenesis, nor even any association with animals.

Serpens spp. strain HBL-112 was isolated by the present inventors from wart tissue of cattle suffering from PDD. The wart tissue was minced and filtered into liquid media as well as by direct inoculation into wells cut into soft agar plates. Incubation of parallel cultures was accomplished at 25-37°C under a variety of atmospheres, such as, 10% CO₂ (candle jar), aerobic, anaerobic and microaerophilic (CampyPak). Final purification of the strain was accomplished by alternate passage between soft agar (0.8%) and standard agar (1.5-2%) plates.

An alternative method for isolating Serpens spp. strain HBL-112 is to mince the wart tissue, place it upon a filter disk (0.45 µm pore size) on a soft agar plate, and incubate it for 2-6 hours under decreased oxygen conditions (candle jar). Removing the filter disk after a short incubation reduces the risk of contamination by swimmers able to swim across the disk but not through it. Using a lowered agar concentration in the agar plate permits rapidly swimming spirochetes (and Serpens spp.) to move through the agar away from lesser mobile bacteria, becoming purified. Repeated sequential passages through the filter/soft agar results in a purified bacterial culture, whether the bacteria is a spirochete or Serpens species, such as Serpens spp., strain

HBL-112.

The rate of movement through the soft agar can be used to distinguish between spirochetes and Serpens spp.. In very soft agar (0.5%) Serpens flexibilis moves 4mm/hour, reaching the edge (from the center) of a 100 mm agar plate in approximately 12 hours while very fast spirochetes move only 0.5 to 0.8 mm/hour. In soft agar (0.8%), S. flexibilis moves 2mm/hour, while Serpens spp. strain HBL-112 moves approximately 1.5 mm/hour.

Microscopic Morphology and Motility

Light microscopy of Serpens spp. strain HBL-112 reveals a poorly staining gram negative rod, often curved, with typically 0.5% to 5% of the cells (up to 100% depending upon media constituents and growth conditions) demonstrating rigid spirals (Figure 5). As shown in Figure 6, the Serpens spp., strain HBL-112 bacteria are highly pleomorphic, with three main forms seen: straight or curved rods, "polliwogs" or spherical cyst-like structures, and rigid spirals. Rods may or may not have sections of rigid spirals interspersed with straight sections. Variations in growing conditions will induce a greater preponderance of one or another form seen under "standard" conditions.

Wet mount phase contrast microscopy of Serpens spp., strain HBL-112 curved and straight rods on the cut edge of a soft agar block reveals the unique and characteristic flexing and serpentine motility reported for Serpens flexibilis. The rods, but not the polliwog or rigid spiral

forms, demonstrate a swimming to serpentine motion, with the serpentine motion and flexibility especially evident under higher viscosity conditions. Direction reversal is rapid, with organisms capable of movement in either direction along their longitudinal axis. Rigid spirals often appear non-motile. "Polliwog" and cyst forms display a swimming/wriggling motility similar to true polliwogs. Using wet mount slides taken from standard agar plates, the morphology of young cultures is predominantly rods. Older cultures exhibit vastly differing rod forms, as well as coccoid and polliwog forms.

With the "soft" agar medium, Serpens spp. are observed by cutting a small agar block out and dicing it onto the slide. Serpens spp. which are observed within the agar or in contact with it exhibit the serpentine motility. Serpens spp. which are washed away from the agar are generally in the rod form, although they exhibit flexing and a spiral-like motility.

A rigid spiral form of Serpens spp. strain HBL-112, as shown in Figure 5, occurs rarely in many media, but becomes more frequent in media containing higher concentrations of sulfur compounds such as cysteine and thioglycollate. When these compounds are added in even higher concentrations, the organism can be converted to nearly 100% spiral form.

-Transmission electron microscopy confirms the three predominant morphological phenotypes. Axial filaments (flagella lying adjacent to the bacterial cell, within a cell membrane) characteristic and essential for identifying

an organism as a spirochete, are not present in Serpens flexibilis nor in Serpens spp., strain HBL-112. Flagella are seen attached terminally on some of the straight and curved rods, and also along the sides of the organism (lateral flagella); the number of flagella on each end (terminal or subterminal) is expected to be approximately 2-4 as shown in Figure 7.

Biochemistry

Biologically pure Serpens spp., strain HBL-112 of the present invention and Serpens flexibilis were characterized by the standard biochemical reactions reported in Tables 1a and 1b below. Table 1a presents data on the biochemical reactions of Serpens flexibilis, Serpens spp., strain HBL-112, of the present invention, as well as three bacterial genera thought to be closely related to the Serpens genus, which currently remain unassigned to a bacterial Family according to Bergey's Manual of Determinative Bacteriology.

Table 1b presents data on the enzyme reactions of Serpens flexibilis, Serpens spp., strain HBL-112, two spirochete strains proposed by CVDLS (California Veterinary Diagnostic Laboratory System) as possible etiological agents for PDD, and a broad sampling of spirochete genera related to the two CVDLS spirochete strains. The "CVDLS isolate" is actually seven strains of spirochete isolated in conjunction with hairy footwart lesions, all having the same enzyme reactions. CVDLS 1-9185 MED is an eighth strain of spirochete which has a different enzyme profile than the

other CVDLS isolates.

[illegible]

Table 1a. Summary of Biochemical Reactions

Biochemical Reaction	HBL #112 Serpens spp.	ATCC 29606 S. flexibilis	Pseudomonas spp.	Alcaligenes spp.	Vibrio hollisae
ONPG	-	-	-	-	-
Arginine dihydrolase	-	-	-	-	-
Lysine Decarboxylase	-	-	-	-	-
Ornithine Decarboxylase	-	-	-	-	-
Citrate as sole C-source	-	-	-	-	-
H ₂ S Production	-	-	-	-	-
Urea hydrolysis	-	-	-	-	-
Tryptophan deaminase	-	-	-	-	-
Indole Production	-	-	-	-	+
VP	-	+	-	-	-
Hydrolyzes Gelatin	-	-	+	-	-
Ferments: Glucose	-	-	-	-	-
Mannose	-	-	-	-	-
Inositol	-	-	-	-	-
Sorbitol	-	-	-	-	-
Rhamnose	-	-	-	-	-
Saccharose	-	-	-	-	-
Melibiose	-	-	-	-	-
Amygdalin	-	-	-	-	-
Arabinose	-	-	-	-	+
Oxidase	+	+	+	+	+
Reduces nitrate to nitrite	+	+	+	+	+
Reduces nitrite to nitrogen	-	+	-	-	-
Motility	+	+	+	+	+
Catalase	+	+	N*	N	N

*N = not available

Table 1 b.
Comparison of Enzyme Reactions

- = no detectable enzyme reaction
 .5 = <5 nanomoles enzyme
 1 = 5 nanomoles enzyme
 2 = 10 nanomoles enzyme
 3 = 20 nanomoles enzyme
 4 = 30 nanomoles enzyme
 5 = 40+ nanomoles enzyme
 + = positive, but no level given

Related Bacterial Spp.

HBL isolate, Strain #112 (1)*

ATCC Strain 29606 (1)

CYDLS isolate (7)

CYDLS 1-9185 MED (1)

Borrelia spp. (6)

Leptospira interrogans (12)

Treponema spp. (oral) (1)

Serpulina spp. (8)

Human intestinal spirochetes (2)

Human & avian spirochetes (4)

Enzyme Composition

Alkaline phosphatase	.5	.5	+	+	.5-2	2-4	2	3-5	1	1-2
Esterase (C4)	2	2	+	+	0-0.5	.5	.5	1-4	1	0-2
Esterase lipase (C8)	4	4	+	+	1	2-3	.5-1	1-4	1	2
Lipase (C14)	1	.5	-	-	-	1-2	-	-	-	-
Leucine arylamidase	3	4	-	-	2-4	3	-	-	-	-
Valine arylamidase	.5	.5	-	-	-	.5-1	-	-	-	-
Cystine arylamidase	-	-	-	-	-	.5-1	-	-	-	-
Trypsin	-	-	-	+	-	-	-	0-2	-	-
Chymotrypsin	-	-	-	+	-	-	-	0-5	-	-
Acid phosphatase	.5	.5	+	+	1-2	3	3-4	3-5	1	2-4
Naphthol-AS-BI-phosphohydrolase	.5	1	+	+	.5-1	.5-2	.5	0-2	-	0-5
α -galactosidase	-	-	-	-	-	.5-2	-	3-5	-	.5-3
β -galactosidase	-	-	+	-	-	1	5	5	1	4
β -glucuronidase	-	-	+	-	-	-	5	3-4	-	-
α -glucosidase	-	-	-	-	-	1	-	2-5	-	.5
β -glucosidase	-	-	-	-	-	1	-	3-5	-	.5
N-acetyl- β -glucosaminidase	-	-	+	-	-	-	4	-	-	.5
α -mannosidase	-	-	-	-	-	-	-	-	-	-
α -fucosidase	-	-	-	-	-	-	1	-	-	-

* (#) = # isolates or strains tested

As shown in Table 1a, Serpens spp. strain HBL-112 of the invention is very weakly catalase positive by peroxide, oxidase positive by spot oxidase testing (Difco Spot-test), and gives the following reactions after forty-eight hours of incubation (at 35-36°C) on API 20E strips (bioMérieux): negative for ONPG (o-nitrophenyl-β-D-Galactopyranoside), negative for arginine dihydrolase, negative for lysine decarboxylase, negative for ornithine decarboxylase, does not use citrate, does not produce hydrogen sulfide from thiosulfate, negative for urease, negative for tryptophan deaminase, does not form indole from tryptophan, does not produce acetoin from pyruvate, does not liquify gelatin, and does not ferment any of the 20E sugars (glucose, mannitol, inositol, sorbitol, rhamnose, sucrose, melibiose, amygdalin, L-arabinose); it does reduce nitrate to nitrite, but not to nitrogen gas.

As illustrated in Table 1a, Serpens spp., strain HBL-112 is distinct from S. flexibilis in that it gives a negative Voges-Proskauer reaction (VP) whereas the S. flexibilis gives a positive reaction. The Voges-Proskauer test is used to determine the bacteria's ability to metabolize pyruvate into acetoin, an intermediary glucose metabolite. Another clear difference is the ability of the Serpens spp., strain HBL-112 to migrate through soft (0.8%) agar plates. S. flexibilis migrates about 2mm/hour whereas Serpens spp., strain HBL-112 migrates about 70-80% as fast. S. flexibilis' faster velocity through soft agar is matched by its higher growth rate in several medias including both

Mueller Hinton broth and TSBA plates.

As shown in Table 1b, Serpens spp., strain HBL-112 gives the following reactions after a four hour incubation at 35-36°C for the enzymes on the API-ZYM (bioMérieux) test strip after forty-eight hours aerobic growth on tryptic soy agar blood plates or in OTI broth or Mueller Hinton broth: weakly positive for alkaline phosphatase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, and valine arylamidase; positive for C4 esterase and C14 lipase; strongly positive for C8 esterase-lipase and leucine arylamidase. The remaining eleven enzymes tested for on this strip were negative. Under the same growth conditions, Serpens flexibilis yields identical results.

Growth parameters

Serpens spp., strain HBL-112, is capable of growth on solid phase standard tryptic soy blood agar (TSBA), Mueller Hinton, and chocolate agars (1.5-2% agar) or soft BSK-H (0.8% agar) plates under aerobic, anaerobic, 10% CO₂ (candle jar), and microaerophilic (BBL Campypak or Campypak Plus) conditions. With more oxygen, the organism has a slightly increased ability to migrate across the top of the agar surface. Growth on TSBA and BSK-H agar in these atmospheres occurs at 25°C, 30°C, and 35-36°C; the temperature range is not however fully defined yet.

Serpens spp., strain HBL-112 is capable of growth suspended in liquid media in modified Eagle's media (MEM),

Mueller Hinton broth, and fluid thioglycollate (FTG) media at 35-36°C. The addition of sterile donor horse serum at 2-5% (v/v) does not appear to affect growth in these medias.

Serpens spp., strain HBL-112 is capable of growth in standard microbiological liquid media at a pH range of 6.8 to 9.4, with optimum growth at approximately pH 7.4.

As used herein, the term, "Serpens spp. strain HBL-112" means bacteria of the Serpens spp. strain HBL-112 having the biochemical reactions set forth in Tables 1a and 1b.

The present invention encompasses the use of Serpens spp. strain HBL-112, Serpens flexibilis, or other Serpens species bacteria, and/or an immunologically active portion thereof, and/or an antigenic epitope substantially cross-reactive with immunologically active portion(s) of Serpens species bacteria to provoke a protective immune response against PDD in ruminant species for the prevention and/or treatment of PDD.

A vaccine containing the bacteria or bacterin may be administered to animals having symptoms of PDD, or administered to animals having no signs of the disease.

The present invention provides methods and compositions for the prevention and/or treatment of PDD in ruminants, such as bovine, ovine and caprine species, comprising an effective amount of Serpens bacteria (live or killed) or an immunologically active portion thereof and an immunologically rational carrier, adjuvant, emulsifier and/or diluent herefor. Suitable Serpens bacteria are Serpens spp. strain HBL-112 and S. flexibilis bacteria,

preferably Serpens spp. strain HBL-112. The killed bacteria may be conveniently prepared by propagation of pure culture Serpens spp. in conventional microbiological media, killing the bacteria by any suitable known method, and standardizing the antigenic mass to an appropriate CFU/ml equivalent. Where live vaccines are desired, the killing step is omitted, but the rest of the formulation proceeds as for the killed suspension. Where the purpose is to prepare a vaccine, suitable carrier(s), adjuvant(s), emulsifier(s), and/or diluent(s) may then be added to the (live or killed) bacterial suspension.

The composition of the present invention for the prevention and/or treatment of PDD may be prepared in a conventional manner by admixing the Serpens spp. killed or live bacterial suspension or an immunologically active portion thereof with an immunologically rational carrier, adjuvant, emulsifier and/or diluent, such as aluminum hydroxide or pharmaceutical grade mineral oil and emulsifier.

It is presently preferred to administer the composition of the present invention by subcutaneous administration, although parenteral or oral administration may be used as well. Oral compositions may incorporate the Serpens spp. bacteria or an immunologically active portion thereof or an antigenic epitope substantially cross-reactive with immunologically active portion(s) of Serpens spp. bacteria in drinking water or feed.

Although the dosage and regimen must in each case be

adjusted, using professional judgment and considering the weight of the animal, generally the dosage will be from about 1×10^8 to about 1×10^{11} CFU/ml, preferably from about 1×10^9 to about 1×10^{10} CFU/ml based upon a 5 ml dose administered subcutaneously. In some instances, a sufficient therapeutic dose can be obtained at a lower dose while in others a larger dose will be required.

Although Figure 1 shows vaccine doses administered at day 0, day 8 and day 35, it is presently preferred to administer two to three doses, the first at day zero, the second dose three to four weeks later and when desirable a third dose may be administered about three to four weeks after the second dose. Suitably, the doses will contain the same amount of bacteria or bacterin.

Parenteral administration can be effected utilizing liquid dosage unit forms such as sterile solutions and suspensions intended for subcutaneous, or intramuscular injection. These are prepared by suspending or dissolving a measured amount of the prepared bacterial suspension in a non-toxic sterile liquid vehicle suitable for injection, such as a sterile aqueous or oleaginous medium. Alternatively, a measured amount of the sterile bacterial suspension is placed in a sterile vial and sealed, or lyophilized and sealed. An accompanying sterile vial or vehicle can be provided for mixing prior to administration. Nontoxic salts and salt solutions can be added to render the injection isotonic. Adjuvants, stabilizers, preservatives

and emulsifiers can also be added.

The present invention provides a method for the diagnosis of PDD and the detection of Serpens antibodies or Serpens antigens using conventional immunoassay methods. Sera from clinically affected or exposed but unvaccinated animals reacts with the Serpens antigen if it contains antibodies to the bacteria. Bound antibodies may be measured. Therefore the Serpens antigen can be used in a diagnostic test for screening of unvaccinated animals for prior exposure to the agent. Similarly, such a diagnostic test may also be used to assess the immune status of a vaccinated animal with respect to the Serpens antigen.

Conversely, antibodies made in and harvested from animals vaccinated with the Serpens bacteria may be utilized for the detection of Serpens bacteria.

A method for determining the presence of PDD antigen in a sample of ruminant tissue comprises administering a Serpens spp. bacteria or Serpens spp. bacterin and/or an immunologically active portion thereof and/or an antigenic epitope cross-reactive with Serpens spp. to the ruminant, harvesting the resultant antibodies or harvesting antibody producing cells and subsequently harvesting antibodies from those cells, binding the antibodies to binding partners and directly or indirectly measuring the binding reaction.

Serpens antigens, or anti-Serpens antibodies, or immunologically active fractions of either, may be used in a process designed for the concentration or purification of the corollary binding partner for use as a reagent.

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A method for determining the presence of PDD antibodies in a sample of ruminant serum may suitably comprise contacting the sample with the Serpens spp. bacteria, preferably Serpens spp. strain HBL-112 bacteria, and detecting antibodies in said sample which bind to said antigen. A method for detecting PDD, or exposure to the Serpens bacteria thereof, via determining the presence of Serpens antibodies in a sample of ruminant serum may suitably comprise incubation of the sample with a solution containing at least one binding partner capable of binding to Serpens antibodies, and directly or indirectly determining the presence of conjugated binding partners in the sample.

The present invention uses serum for the samples and Serpens antigen as the respective binding partner, however the specific binding reaction may be utilized to detect either partner, hence other samples such as tissue sections, cell smears and parts thereof, or environmental samples, Serpens per se or fractions thereof may be studied using this method.

Either binding partner may incorporate one or more detectable markers such as a radioisotope, metal or fluorochrome, or may be detected indirectly, as through the use of conjugated enzyme with substrate detection.

Alternatively, in cases where neither of the primary binding partners incorporates a marker or other means for direct detection, secondary binding partnerships may be formed for subsequent detection either using Serpens group

antigens or their complementary antibodies and/or anti-Serpens antibodies or through the use of binding partners which are not Serpens related, (such as avidin-biotin) but which may be used directly or indirectly for the detection of the primary binding partners.

Subsequent detection and quantification of the above markers, binding partnerships or other measurable medium may be by any conventional means appropriate to the methodology.

Alternatively, detection of Serpens in a sample can be made by means of directly or indirectly measuring physical features distinct to or characteristic of Serpens. Such measurements can include detection and measurements of compounds, or compound mixtures, including lipids, proteins or nucleic acids such as used in DNA amplification and identification techniques, or chromatographic separation and identification techniques such as gas or liquid chromatography.

In a further aspect of the invention, there is provided a diagnostic kit for use in performing the method according to the invention, which kit comprises Serpens antigen and one or more binding partners. The diagnostic kit may further include reagents required for sample preparation and optionally reagents for the detection of the bound antibody.

The present invention is illustrated in terms of its preferred embodiments in the following examples. All temperatures are in degrees Centigrade and all parts and proportions are by weight, unless otherwise noted.

Example 1

Whole-cell Serpens spp. strain HBL-112, Serpens flexibilis or other Serpens spp. bacterins (killed cultures) are prepared by propagating bacterial cells in a standard microbiological media, killing the cells with formaldehyde, washing with sterile saline to remove cell debris, unused media components, bacterial waste products and the like, and suspending the bacterin in sterile phosphate buffered saline with 10% (v/v) aluminum hydroxide (adjuvant) and 0.01% thimerosal (preservative).

Example 2

Whole-cell Serpens spp. strain HBL-112 or Serpens flexibilis or other Serpens spp. bacterins (killed cultures) are prepared by propagating bacterial cells in a standard microbiological media, killing the cells with formaldehyde, washing with sterile saline to remove cell debris, unused media components, bacterial waste products and the like, and emulsifying the bacterin in sterile phosphate buffered saline with 25% (v/v) pharmaceutical grade mineral oil and emulsifiers (adjuvant) and 0.01% thimerosal (preservative).

Example 3

A non-virulent Serpens spp. or other apathogenic bacterial strain bearing cross-protective Serpens like antigens is propagated under standardized conditions (for example: using one of several conventional microbiological medias with a pH between 6.8 to 9.4, incubated at a temperature of approximately 25-37°C under one of a variety of atmospheres for a period of a few days to several weeks)

ascertained to be a pure culture by suitable testing such as microscopic and colonial morphology, velocity through soft agar, characteristic motility under phase contrast microscopy, and/or biochemical testing and is then harvested. The harvested cells are washed, suspended in a sterile vehicle (for example, buffered saline) containing any conventional cryoprotectant typically used in vaccine manufacturing, filled into sterile vials and preserved by either freezing or lyophilization. The preserved material is thawed or reconstituted for administration either subcutaneously, orally or parenterally to ruminants at the appropriate dosage.

Example 4

A total of 76 dairy cows with active, untreated lesions of PDD were enrolled in a treatment-based vaccine trial. Fifty animals were from the first milking string (highest producing cows), and twenty-six were from the hospital string. Since the hospital cows were likely undergoing treatment for mastitis which may have altered their immunological profile, only the first string cows were sampled and followed serologically.

All animals were scored for lameness and number of feet involved at the start of the trial; within lameness/foot groupings, each cow was randomly assigned to receive either vaccine or placebo for the trial. On the first day and approximately twice weekly thereafter each animal's feet were cleaned with plain water sprayed under moderate pressure from a hose-end sprayer and evaluated for presence,

The reason the control group begins to show improvement after day 40 (Figure 1), was that an oxytetracycline footbath was administered to the vaccinate and control cows. However, the vaccinates maintained their statistically significant advantage over controls in wart area reduction despite the improvement in controls attributable solely to the oxytetracycline footbath.

Three animals showing signs of lameness did not have evidence of lesions upon enrollment. When they did not develop lesions by eight weeks into the trial, they were dropped from the lesion analysis portions of the trial. Two of these animals did provide some useful information: neither was serologically positive on ELISA testing using Serpens spp. strain HBL-112 as the antigen, suggesting no prior exposure to the epitopes on this organism. That these non-lesion bearing animals did not recognize the Serpens spp. strain HBL-112 in an ELISA, while lesion-bearing nonvaccinates (controls) did, strongly suggests that a Serpens spp. bacteria is involved in the pathogenesis of the PDD lesion.

Figures 2 and 3 show effects occurring prior to any effect from the antibiotic footbath; the clinical effects seen in vaccinates (and lack thereof in controls) are thus attributable solely to the use of Serpens spp. strain HBL-112 bacterin. Further, limiting the data in the bar charts to results seen only through day 49 underreports the clinical improvement seen following the third dose of vaccine administered on day 36.

the twenty-two vaccinates showed a fairly large increase in titer (.441 increased to .560) in response to vaccination; one animal showed a small decrease in titer.

Table 2 below, presents Type III Sums of Squares tables for dependent variables wart area and titer, showing statistically significant differences between vaccinates and controls in response to vaccination. While the herd as a whole improved significantly in lesion size (wart area) between the start of the trial and after 49 days (p value = 0.0420), the most significant effect is attributed to the vaccinates: p value = 0.0155 for the interaction of pre-post with vaccination status (=group). The change in titer seen in the herd as a whole between the start of the trial and at 49 days is solely attributable to the increases seen in vaccinates (p value for pre-post by group is highly significant at p value = 0.0001 vs pre-post effects alone at p value = 0.2682).

Table 3 below, presents a series of pre- and post-vaccination Means tables for the dependent variables examined in the analysis of the vaccine trial.

Of the factors measured, only total wart area shows statistically significant (@ $p < 0.05$) differences between vaccinates and controls. Vaccinates definitely did not develop as large lesions, nor did they remain lesioned as long as controls. See Figures 1-3.

TABLE 2

Type III Sums of Squares

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
Group	1	185.306	185.306	2.005	.1643
Milk ProductionMilk Pro...	1	5.012	5.012	.054	.8170
Subject(Group)	41	3789.260	92.421		
pre/post	1	114.663	114.663	4.474	.0420
pre/post * Group	1	166.998	166.998	6.516	.0155
pre/post * Milk Product...	1	102.986	102.986	4.018	.0533
pre/post * Subject(Gro...	33	845.727	25.628		

Dependent: Lesion Area

Type III Sums of Squares

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
Group	1	.057	.057	1.774	.1902
Milk ProductionMilk Produ...	1	.001	.001	.037	.8492
Subject(Group)	41	1.310	.032		
pre/post	1	.004	.004	1.268	.2682
pre/post * Group	1	.099	.099	35.402	.0001
pre/post * Milk Productio...	1	8.995E-5	8.995E-5	.032	.8584
pre/post * Subject(Group)	33	.092	.003		

Dependent: Titer

TABLE 3

Means Table
Effect: pre/post * Group
Dependent: Lesion Area

	Count	Mean	Std. Dev.	Std. Error
pre, vaccinate	20	10.850	7.252	1.622
pre, control	23	11.504	6.614	1.379
post, vaccinate	15	8.220	9.115	2.353
post, control	22	15.345	9.005	1.920

Means Table
Effect: pre/post * Group
Dependent: Lesion #

	Count	Mean	Std. Dev.	Std. Error
pre, vaccinate	20	1.850	.988	.221
pre, control	23	2.087	1.276	.266
post, vaccinate	15	2.333	1.397	.361
post, control	22	3.000	1.574	.335

Means Table
Effect: pre/post * Group
Dependent: Feet Affected

	Count	Mean	Std. Dev.	Std. Error
pre, vaccinate	20	1.750	.967	.216
pre, control	23	1.783	.736	.153
post, vaccinate	15	2.067	1.100	.284
post, control	22	2.227	.813	.173

TABLE 3 CONT.

Means Table
Effect: pre/post * Group
Dependent: Lameness

	Count	Mean	Std. Dev.	Std. Error
pre, vaccinate	20	1.400	.883	.197
pre, control	23	1.261	.810	.169
post, vaccinate	15	.533	.516	.133
post, control	22	.818	.733	.156

Means Table
Effect: pre/post * Group
Dependent: Titer

	Count	Mean	Std. Dev.	Std. Error
pre, vaccinate	20	.441	.134	.030
pre, control	23	.460	.123	.026
post, vaccinate	15	.560	.173	.045
post, control	22	.444	.130	.028

Example 5

The same antigen present in the vaccine, Serpens spp. strain HBL-112 bacterin, is also used in an ELISA test to monitor serological response to vaccination. Antigen (a 5% suspension of concentrated killed, washed whole cells, Serpens spp. strain HBL-112 bacteria, approximately 5×10^8 cells/ml, in a coating buffer, a sodium carbonate/bicarbonate buffer at pH 9.6) is placed in the wells of a 96-well microtiter plate overnight at room temperature. The plate is gently washed using a wash buffer, (a sodium phosphate buffer containing a detergent such as Tween or Triton (pH 7.5)). A bovine serum sample (primary antibody) is added and incubated in the plate at room temperature for one hour. The plate is gently washed again using wash buffer to get rid of unbound antibodies. A secondary antibody made of an anti-cow antibody, such as from goat, conjugated with alkaline phosphatase is incubated in the plate for one hour at room temperature. The plate is gently washed again using wash buffer. The plate is then incubated with p-nitrophenyl phosphate substrate in a 10% diethanolamine buffer (pH 9.8) and allowed to develop at room temperature until the maximum well O.D. is approximately 0.8 to 1.0, no stopping agents are used. The alkaline phosphatase enzyme attached to the secondary antibody converts the p-nitrophenyl phosphate substrate and turns the clear solution in the plate yellow. Binding of the primary antibody, secondary antibody and hence the strength

of the O.D. reading is proportional to the amount of antibody present in the cow serum against the Serpens spp. strain HBL-112 antigen.

This method is essentially standard for ELISA, and any other ELISA method and numerous variations in this procedure would be expected to produce similar results. The procedure does differ from typical ELISA methods in that the incubation steps are done at room temperature. The purpose of this is only to reduce intrawell variation due to temperature induced edge effects.

Sera from unvaccinated animals binds to the Serpens spp. strain HBL-112 bacteria antigen if it contains antibodies for the bacteria.

Example 6

It is often desirable to use a multivalent (multiple antigen) vaccine preparation to minimize the number of times an animal must be injected. Using a single pharmaceutical preparation which incorporates multiple antigens minimizes pain and risk of infection (abscesses at the injection site) for the animal, and decreases labor costs and risk of human injury (handling injuries and/or accidental self-injection) for the owner and their employees.

The same antigen used alone to produce the vaccines described in Examples 1 and 2 may also be used in combination with either antigens to produce a multivalent vaccine useful for treating and/or preventing other bacterial infections of ruminants in addition to PDD. The

flexibility of the Serpens spp. vaccine schedule would permit virtually any other currently used ruminant vaccine antigen to be advantageously incorporated with Serpens spp. in a vaccine for simultaneous administration.

Alternatively, the Serpens spp. antigen could be incorporated into any other ruminant pharmaceutical preparation administered by any method other than the intravenous route.

For example, strains of anaerobic bacteria such as members of the Bacteroides and/or Fusobacterium genera which are causative agents of the condition known as footrot in cattle and sheep may be incorporated with Serpens spp. antigens in a pharmaceutical preparation which when administered to susceptible ruminants will prevent both PDD and footrot. In another example, prevention of PDD in dairy cows may optimally require vaccination with Serpens spp. antigens during the non-milking "dry" period since the outbreaks of disease appear to peak during the first few months after lactation starts. Since many other vaccines are administered to the cow during this period to provoke high maternal antibody titers for transmission to the calf via colostrum, the Serpens spp. antigen may be advantageously administered to the cow with these other vaccine antigens during this dry period.

Alternatively, certain other antigens are known to have general immune-stimulatory properties, such as the "superantigen" present in members of the Staphylococcus genus or the "core antigen" of certain members of the

Claims

1. Biologically pure Serpens spp. strain HBL-112.
2. A pharmaceutical composition for preventing and/or treating Papillomatous Digital Dermatitis in ruminants comprising a therapeutically effective amount of Serpens spp. bacteria or Serpens spp. bacterin and/or an immunologically active portion thereof and/or an antigenic epitope cross-reactive with Serpens spp. and a veterinerally acceptable diluent or a carrier.
3. The composition of claim 2 wherein said ruminant has symptoms of Papillomatous Digital Dermatitis.
4. The composition of claim 2 wherein said ruminant has no symptoms of Papillomatous Digital Dermatitis.
5. The composition of claim 2 comprising Serpens spp. strain HBL-112 or Serpens spp. strain HBL-112 bacterin.
6. The composition of claim 2 comprising Serpens flexibilis or Serpens flexibilis bacterin.
7. A method of preventing and/or treating Papillomatous Digital Dermatitis in ruminants comprising administering to the ruminant a therapeutically effective amount of Serpens spp. bacteria or Serpens spp. bacterin and/or an immunologically active portion thereof and/or an antigenic epitope cross-reactive with Serpens spp..
8. The method of claim 7 wherein said ruminant has symptoms of Papillomatous Digital Dermatitis.
9. The method of claim 7 wherein said ruminant has no symptoms of Papillomatous Digital Dermatitis.
10. The method of claim 7 wherein Serpens spp. strain HBL-

112 or Serpens spp. strain HBL-112 bacterin is administered to said ruminant.

11. The method of claim 7 wherein Serpens flexibilis or Serpens flexibilis bacterin is administered to said ruminant.

12. A method for determining the presence of PDD antibodies in a sample of ruminant serum comprising contacting said sample with an antigen selected from the group consisting of bacteria or bacterin of the Serpens genus or an immunologically active portion thereof and/or an antigenic epitope cross-reactive with Serpens spp. and detecting antibodies in said sample which bind to said antigen.

13. A method for determining the presence of PDD antibodies in a sample of ruminant serum comprising contacting the sample with a solution containing at least one binding partner capable of binding to the Serpens spp. strain HBL-112 or an immunologically active portion thereof and/or an antigenic epitope cross-reactive with Serpens spp. strain HBL-112, removing unbound binding partner and determining the presence of bound binding partner in the sample.

14. A method for determining the presence of anti-Serpens spp. antibodies in a ruminant comprising contacting Serpens spp. antigen with a serum sample from a ruminant, adding an anti-ruminant antibody having an enzyme attached, and adding a substrate with which said enzyme can convert to a readily measured product.

15. A method for determining the presence of PDD antigen in a sample of ruminant serum comprising contacting said sample

with an antibody selected from the group comprising the Serpens genus and detecting antigen in said sample which bind to said antibody.

16. A diagnostic kit for use in a method according to claim 14 for determination of the presence of PDD antibodies said kit comprising antigen and one or more binding partners.

17. The kit of claim 16 wherein reagents for sample preparation and reagents for detection of bound antibody are included.

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2072-2073	2073-2074
2073-2074	2074-2075
2074-2075	2075-2076
2075-2076	2076-2077
2076-2077	2077-2078
2077-2078	2078-2079
2078-2079	2079-2080
2079-2080	2080-2081
2080-2081	2081-2082
2081-2082	2082-2083
2082-2083	2083-2084
2083-2084	2084-2085
2084-2085	2085-2086
2085-2086	2086-2087
2086-2087	2087-2088
2087-2088	2088-2089
2088-2089	2089-2090
2089-2090	2090-2091
2090-2091	2091-2092
2091-2092	2092-2093
2092-2093	2093-2094
2093-2094	2094-2095
2094-2095	2095-2096
2095-2096	2096-2097
2096-2097	2097-2098
2097-2098	2098-2099
2098-2099	2099-2100
2099-2100	2100-2101
2100-2101	2101-2102
2101-2102	2102-2103

Response to Footwart Vaccine

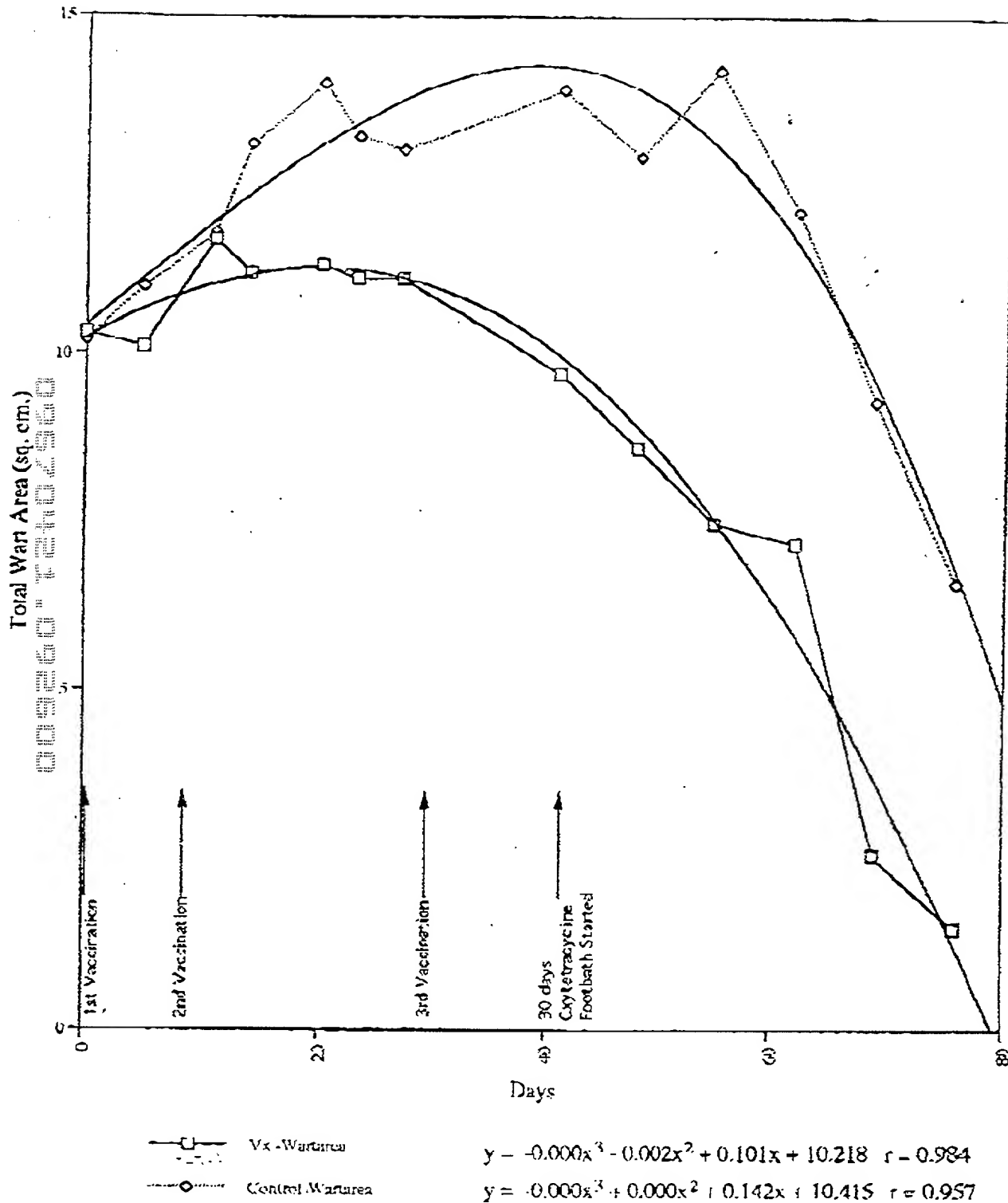
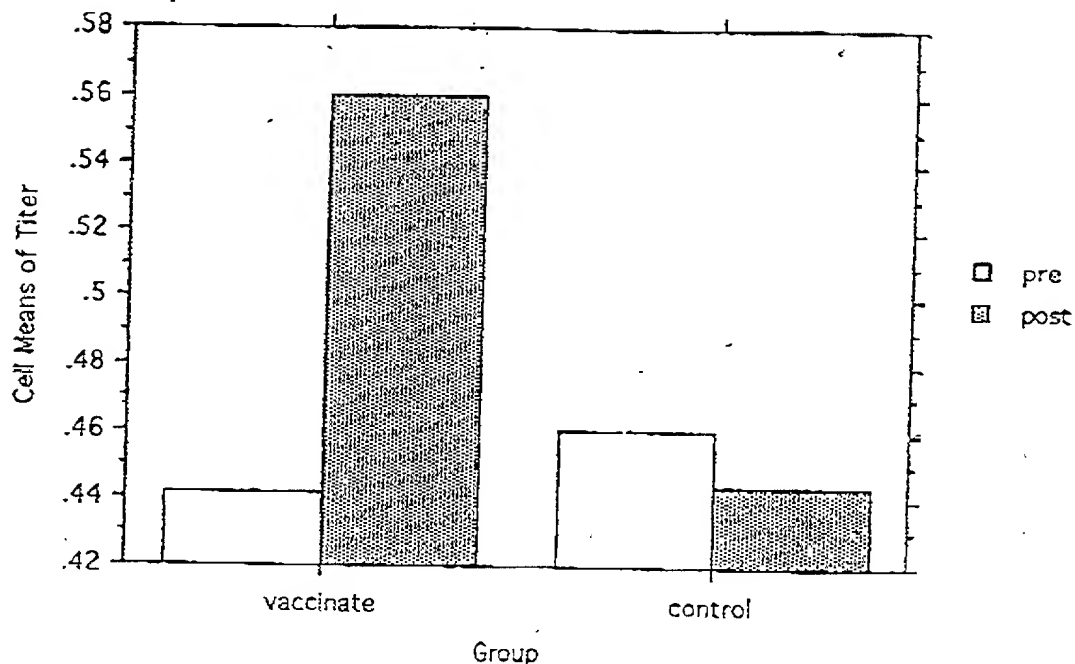


FIGURE 1

Interaction Bar Chart
Effect: pre/post * Group
Dependent: Titer



Games-Howell
Effect: Group
Error term: Type III sum of squares for Subject(Group)
Dependent: Titer
Significance level: .05

	Vs.	Diff.	Crit. diff.
control	vaccinate	.040	.065

None were significantly different at this level.

Helmholtz Comparison 1
Effect: pre/post
Dependent: Titer

	Cell Weight
pre	1.000
post	-1.000

df 1
Sum of Squares .053
Mean Square .053
F-Value 18.950
P-Value .0001

FIGURE 3

Figure 4 (b)

09670424.092600



FIGURE 5



FIGURE 6

36 1412
+ 022000

No. 8899 P. 20/20

FIGURE 7

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